pH Responsive polyurethane (core) and cellulose acetate phthalate (shell) electrospun fibers for intravaginal drug delivery

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Abstract

In this study we present the use of co-axial electrospinning to produce core-shell composite micro-/nano- fibers of Polyurethane (PU) and Cellulose acetate phthalate (CAP). The designed fibers possess enhanced mechanical properties with a tensile strength of 13.27±2.32 MPa, which is a clear improvement over the existing CAP fibers that suffer from a poor mechanical strength (0.2±0.03 MPa). The CAP imparts pH responsiveness to the core-shell structure giving the fibers potential for "semen sensitive" (intravaginal) drug delivery.

Key words: core-shell electrospinning; cellulose acetate phthalate; polyurethane; intravaginal drug delivery

1. Introduction

In recent years, the acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus (HIV), has become a serious threat to human health. Until now, about 33 million people are infected by HIV (Mamo, Moseman et al., 2010). The prevalent sources of transmission of this virus are mainly through sexual contact, transfusion of contaminated blood or medical products such as syringes. The prevention of this pandemic disease has been better than its cure since the HIV virus presents varied genetic variability retarding the development of suitable vaccines. Hence, the most common methods of prevention of this deadly disease have been the use of condoms and microbicides. However, in most of the developing countries, the use of condoms and microbicides has been restricted due to social taboos (Date and Destache, 2013). Clearly, alternate strategies to combat or prevent HIV spreading remain highly needed.

In our previous work (Huang, Soenen et al., 2012), we designed cellulose acetate phthalate (CAP) fibers by electrospinning. CAP is being intensively used as pharmaceutical excipient to coat capsules and tablets, with the aim to avoid drug release in the acidic stomach while allowing drug release in the more alkaline intestine. We showed that electrospun CAP-fibers instantaneously dissolve upon contact with (human) semen. Indeed, as the pKa of CAP equals 5.28 (Rando, Obara et al., 2006), it is expected to be minimally soluble in healthy vaginal flora but highly soluble when exposed to semen with a pH of approximately 7. Note that CAP itself has gained attention as well for its anti-HIV effect due to its ability to induce conformational changes in the HIV glycoproteins gp41 and gp120 (Neurath, Strick et al., 2002). We thus suggested that CAP-fibers could have potential for 'semen triggered' delivery of (anti-viral) drugs.

The previously designed electrospun CAP-fibers showed, however, a (very) poor mechanical

48 performance, resulting in fractures, which would clearly limit their intravaginal use. The current
49 research effort has been dedicated to overcome this drawback through the design of core-shell fibers
50 by co-axial electrospinning. Polyurethane (PU) is used as core to enhance the mechanical properties
51 of the fibers; Due to the thermodynamic incompatibility between soft and hard segments in the PU
52 main chains (Skarja and Woodhouse, 1998; Hong, Guan et al., 2010), PU possesses excellent
53 mechanical properties, including high elongation at break and mechanical strength (Jiang, Greiner et
54 al., 2013; Jiang, Duan et al., 2014). The shell of the fibers consists of CAP which is widely used in
55 pharmacy and biomedicine and is highly biocompatible (McDevitt, Woodhouse et al., 2003; Guan,
56 Fujimoto et al., 2005; Tseng, Tang et al., 2005; Shau, Tseng et al., 2006; Hashizume, Fujimoto et al.,
57 2010). Also note that co-axial electrospinning is used in the biomedical field for various purposes, such
58 as to preserve unstable biological agents and viruses (Salalha, Kuhn et al., 2006), to prevent
59 decomposition of unstable compounds (Peh, Lim et al., 2015) and to achieve sustained drug release
60 (Qi, Guo et al., 2010). Also, Ball (Ball, 2014) showed that fibers obtained by co-axial electrospinning
61 allowed the sustained release of microbicides from fibers. They could deliver the compounds over
62 multiple timescales and composite microbicide fabrics were created to provide both rapid and
63 sustained drug release from a single device.
64
65 **2. Materials and methods**
66 **2.1 Materials**
67 Rhodamine B, Polyurethane (PU, M_w = 100,000 g/mol), 3-(2-Beenzothiazolyl)-7-(diethylamino)
68 (Coumarin 6), Tetrahydrofuran (THF), 2-Methoxyethanol, Dimethylformamide (DMF) and Acetone
69 were purchased from Daguangming (Nanjing, China). Cellulose acetate phthalate (CAP, M_w = 60,000

g/mol) was purchased from Sigma-Aldrich (Steinheim, Germany). MTT kits were purchased from Aladdin (Shanghai, China). L929 cells were purchased from BioCambridge (Nanjing, China).

2.2 Electrospun PU and CAP fibers

PU (core)/CAP (shell) fibers were obtained by coaxial electrospinning a CAP solution (25%, w/v) using 2-Methoxyethanol/acetone/distilled water (1:0.85:0.15, v/v/v) as solvent (Olaru and Olaru, 2010) and a PU solution (14%, w/v) using a THF/DMF (1:1, v/v) as solvent. Rhodamine B was added to the PU solution prior to electrospinning. PU and CAP solutions were filled in two individual syringes and electrospun by one coaxial electrospinning needle with a flow rate of 0.5 ml/h for PU and 3 ml/h for CAP. The fibers were collected on a metal plate. The distance between the syringe needle and the metal plate was 12 cm. As a control, pure CAP and PU electrospun fiber mats were also prepared using the (same) conditions as used for coaxial electrospinning. All fiber mats were vacuum dried during 24h at 40 ^{o}C.

2.3 Cell culture

L929 cells were maintained in 10% fetal bovine serum (FBS) supplemented Dulbecco's modified Eagle's medium (DMEM, Nanjing, China) and cultured in a humid atmosphere at 37 ^{o}C with addition of 5% CO_2. When the confluence reached 80% the cells were passaged. Cells were feed with fresh medium three times a week.

2.4 Cytotoxic activity

To test the viability of cells exposed to electrospun fiber mats, cells were seeded in 96-well plates

(6000 cells/well). We used DMEM medium to make extracts from the fiber mats (1 mL DMEM was used to extract 0.5 cm^2 fiber mats). The fiber mats were submerged into DMEM at 37 ^oC for 72 h. After centrifugation, the supernatant ('extract') was filtered (using 0.22 μm filters); subsequently the extracts were diluted in PBS. The cells were treated with (diluted) extracts at 37°C for 24h. After incubating the cells with the extract, the extract was removed and the cells were washed twice with PBS. Next, fresh medium containing 5 mg/ml of MTT reagent was added to the cells and incubated for 4 h at 37°C. Following this incubation, the medium was carefully removed and the formazan crystals were dissolved by incubation with 180 μl DMSO on a shaker for 10 min. Finally, the absorbance was measured with a microplate reader at 570 nm. The percentage of viability of the cells was then calculated by comparison with untreated cells representing 100% viability (so 'named relative growth rate', RGR). The RGR was defined as: $RGR=A_e/A_p\times100\%$ (1)

A_e being the absorbance measured in the experimental groups, A_p being the absorbance measured in case DMEM medium was used.

2.5 Rhodamine release study

Rhodamine B loaded fibers were electrospun and air-dried; Therefore 1mg Rhodamine B was dissolved in 0.78ml CAP solution used for electrospinning. We dispersed 5 mg of Rhodamine B-loaded composite fiber mats in Eppendorf tubes filled with 1 ml of respectively pH 7.4 PBS (which served as 'simulated human semen', SHS) or pH 4.2 solution ('simulated vaginal fluid', SVF). The release of Rhodamine B from the coaxial fibers was studied by measuring the fluorescence of the supernatant (excitation light: 528 nm, emission light: 550 nm). Rhodamine B standard curves (in PBS and SVF); (concentration range between 0.001 and 0.01 mg/ml) were used to determine the concentration of the

114 released Rhodamine B.

115

116 **2.6 Characterization of the fibers**

117 SEM images of the coaxial fibers were recorded by Field Emission SEM (JSM-7600F, Japan) operated
118 at an acceleration voltage of 15 kV. TEM images were captured with a JEM-2100 (Japan) transmission
119 electron microscope. The acceleration voltage was 100 kV. Rhodamine B and coumarin 6 were added
120 to stain respectively the PU solution (Red) and the CAP solution (Green). Fluorescent images of the
121 coaxial fibers were recorded by laser scanning confocal microscopy (LSCM, LSM710, Zeiss,
122 Germany). TGA of the electrospun fiber mats was performed in N_2 from 30 ^oC to 600 ^oC at 5 ^oC min^{-1}
123 using a universal V4.5A TA instrument. The tensile tests were carried out by a universal tensile tester,
124 equipped with a load cell which had a maximum load of 50 N and a resolution of 0.001 N. The samples
125 (20 mm × 6 mm) were stretched at a speed of 5 mm/min while the gauge length was set to 10 mm. The
126 average thickness of the samples was measured making use of a screw micrometer.

127

128 **3. Results and discussion**

129 **3.1 Coaxial electrospun microfibers**

130 To overcome the poor performance of "pure" CAP electrospun fibers, coaxial electrospinning was
131 performed to incorporate PU into the core of coaxial fibers. **Figure 1A-E** shows the morphology of
132 pure CAP fibers, pure PU fibers and coaxial fibers. Both the pure CAP fibers and PU fibers possess
133 smooth surfaces (**Fig. 1A** and **1B**). The PU/CAP coaxial fiber is shown in **Fig. 1C**. The diameter of the
134 fiber was above 1 μm and the surface was smooth. The LSCM image confirmed the coaxial structure
135 of the PU/CAP fibers (**Fig. 1D**); the fiber shell was dyed green (CAP) while the fiber the core was

dyed red (PU). The TEM image (**Fig. 1E**) showed PU was wrapped completely in the core while there was a distinct boundary between the core and shell. TGA curves of electrospun fibers are shown in **Fig. 1F**. Pure PU fibers were stable until 300 ºC while a three step weight loss was observed for the coaxial electrospun fibers and pure CAP electrospun fibers. As the amount of PU only accounted for 28 wt% in the coaxial fibers, the coaxial fibers showed a similar decomposition trend as pure CAP fibers, but the thermal stability was in-between pure CAP and pure PU fibers.

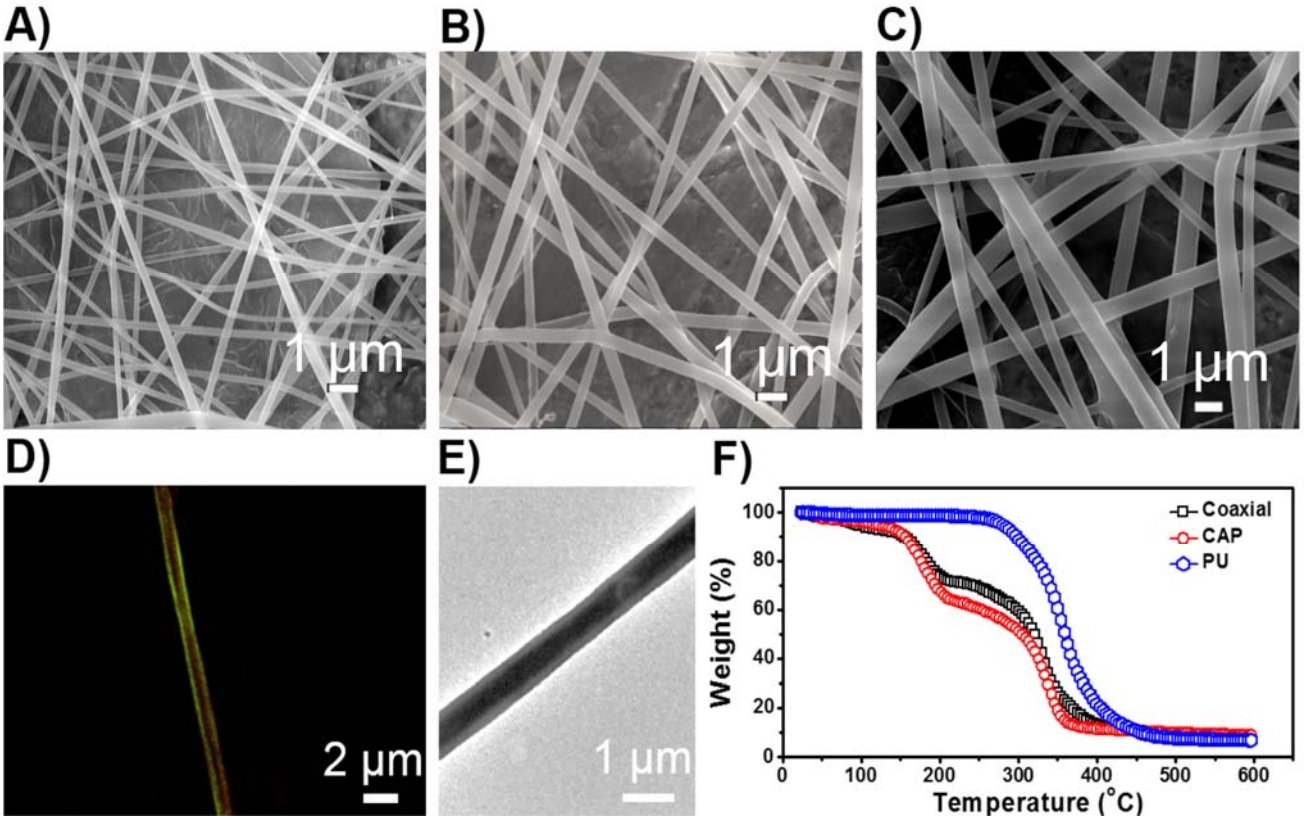

Figure 1. Characterization of the structure of the fibers. SEM images of A: pure CAP fibers; B: pure PU fibers; C: PU/CAP coaxial fibers; D: confocal image of PU/CAP coaxial fiber (Green: CAP; Red: PU); E: TEM of PU/CAP coaxial fibers; F: TGA of electrospun PU/CAP coaxial fibers (28 wt% PU), pure CAP and pure PU fibers.

3.2 Mechanical properties of PU/CAP coaxial microfibers

Electrospun PU fibers have been reported to have excellent mechanical properties. Jiang et al. reported that single electrospun PU fibers possess a tensile strength of 283 MPa and an elongation at break of 589 % which suggest electrospun PU fibers to be good reinforcements and toughening materials (Jiang, Duan et al., 2014). However, electrospun CAP fibers showed poor mechanical properties, which limit their applications. Therefore, in this work, PU was incorporated into CAP electrospun fibers by coaxial electrospinning to improve the mechanical performance of CAP fibers. As shown in **Fig. 2**, pure CAP and pure PU fiber mats exhibit a tensile strength of 0.2±0.03 and 15.2±1.18 MPa, respectively. For coaxial PU/CAP fiber mats (28% PU), the tensile strength reached 13.27±2.32 MPa, which is about 65 times that of pure CAP fibers. When the PU content increased to 33 wt%, the tensile strength decreased however to 11.7 MPa. Elongation at break could be used to evaluate the flexibility of the materials. Pure CAP electrospun fibers showed an elongation at break of about 4%. The incorporation of PU led to significant increase in the elongation at break to about 14%, which is 250% higher than that of pure CAP fibers.

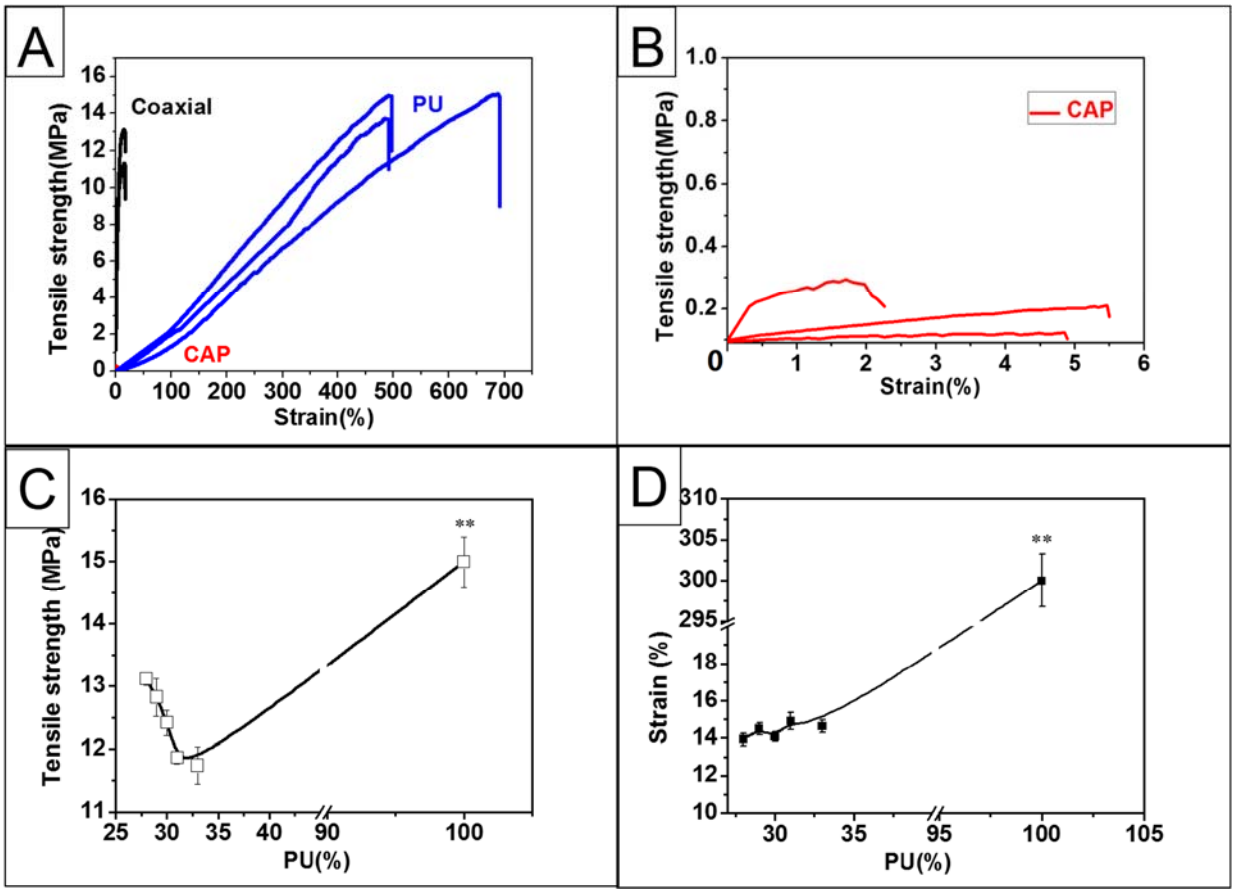

Figure 2. (A) Tensile strength of coaxial fibers containing 28% (w/w) PU, pure PU fibers and pure CAP fibers; (B) Zoom in on the tensile strength of pure CAP fibers; (C)Tensile strength of PU/CAP coaxial fibers with different mass ratio of PU (28%, 29%, 30%, 31%, 33%, 100%); (D) Strain at break of coaxial fibers with different mass ratio of PU (28%, 29%, 30%, 31%, 33%, 100%), **significant at $p < 0.01$

3.3 Cytotoxicity assessment

The result of the cytotoxicity assessment is shown in **Fig. 3**. 100% (Group E) in the x-axes corresponds to the undiluted extract (see section 2.4) while 75% (v/v)(Group D), 50% (v/v)(Group C) and 25% (v/v) (Group B) refer to diluted extracts. Phenol was used as (positive) control sample (Group F). Clearly, the extracts obtained from the fibers mats did not significantly influence the cell growth.

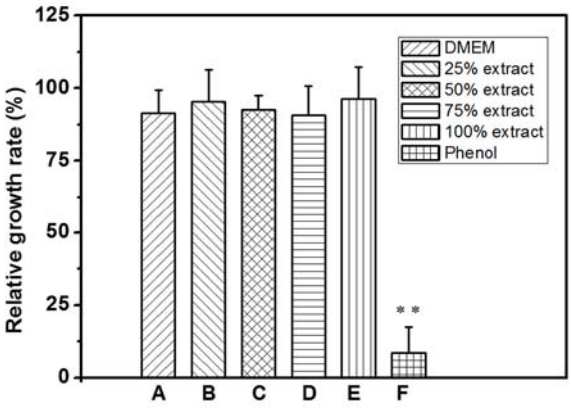

Figure 3. Cytotoxicity assessment of electrospinning PU/CAP fiber mats (28wt% PU), **significant at $p < 0.01$

3.4 Rhodamine release study

To investigate whether the coaxial PU/CAP fibers show pH dependent release features, fibers were exposed to pH 4.2 and pH 7.4, mimicking the pH of the simulated vaginal fluid (SVF) and semen respectively. Note that the fluorophore Rhodamine B was loaded in the CAP shell of the fibers. As can be seen from the micrographs in **Fig. 4**, at pH 4.2 the PU/CAP fiber mats remain loaded with Rhodamine B, even after 3 h. In contrast, the fluorophore was immediately released from the fibers at pH 7.4. Fluorescence measurements (**Fig. 5**) confirmed that coaxial PU/CAP fibers retained Rhodamine B at acidic (vaginal) pH while they released it rapidly (within 1 minute) at pH 7.4. In agreement, we observed by dissolution tests that the coaxial fibers were completely dissolved in pH 7.4 PBS in 1 min. By electrospinning three-dimensional fiber mats with a large specific surface area are obtained which facilitates water absorption and release of the compound encapsulated from the CAP-shell into the water.

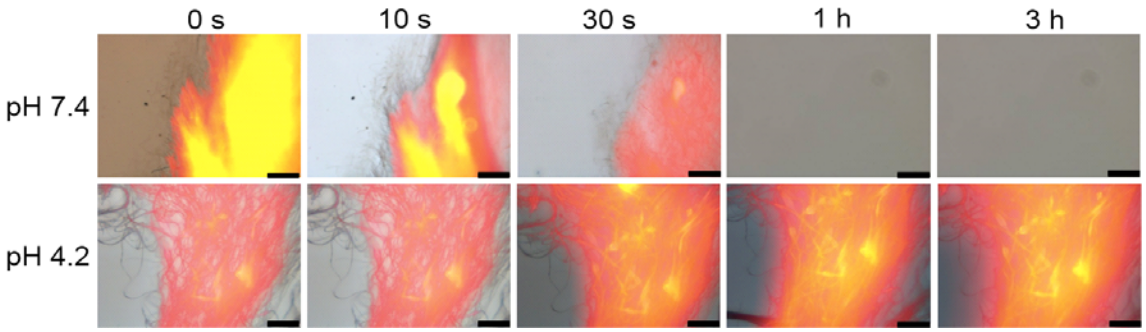

Figure 4. Fluorescence microscopy on Rhodamine B-loaded PU/CAP coaxial fibers (28% w/w PU) dispersed in respectively SVF and PBS. Scale bar = 50 μm.

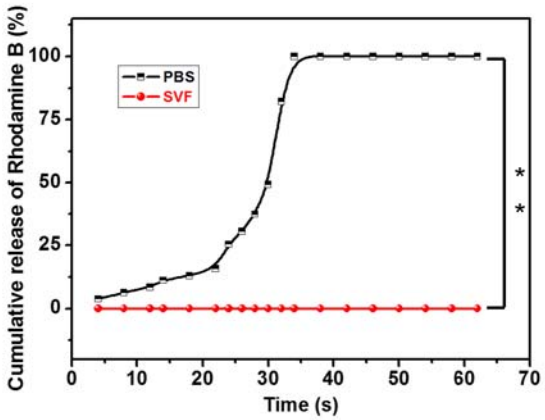

Figure 5. Release profile of Rhodamine B from electrospun fibers in respectively SVF and PBS (appropriate standard curves of Rhodamine B in SVF and PBS were used to calculate the concentration of released Rhodamine B) , **significant at $p < 0.01$

4. Conclusions

In this study we prepared and characterized PU/CAP coaxial fibers with improved mechanical properties and investigated their potential as semen sensitive delivery system. We observed that,

compared to previously reported CAP fibers, the coaxial structure of PU/CAP fibers significantly improved the mechanical strength of the fibers. Our data show that the coaxial fibers remain intact in SVF at pH 4.2 while they dissolve very rapidly in PBS at pH 7.4. Extracts obtained from the fiber mats did not induce cytotoxicity. The core-shell PU/CAP coaxial fibers showed an outspoken pH responsive release of Rhodamine which allows us to conclude that PU/CAP coaxial electrospun fibers mats are promising for pH responsive drug delivery, especially in the context of semen sensitive drug release.

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